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(54) Title: USE OF PROTEIN CONFORMATION FOR THE PROTECTION AND RELEASE OF CHEMICAL COMPOUNDS

(57) Abstract

The invention is directed to a method of protecting a chemical compound from degradation comprising combining the chemical compound with an amino acid polymer or carbohydrate polymer. A pharmaceutical composition comprising an active ingredient that has been combined with an amino acid polymer and a pharmaceutically acceptable excipient has been described. A method of protecting a chemical compound from degradation comprising manipulating the higher order structure of a synthetic protein and combining said chemical compound with the protein has been described. A method of controlling the release of a chemical compound comprising manipulating the higher order structure of a synthetic protein and combining said chemical compound with the protein, as well as by manipulating the length of the protein, has been described.

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USE OF PROTEIN CONFORMATION FOR THE PROTECTION AND RELEASE OF CHEMICAL COMPOUNDS

BACKGROUND OF THE INVENTION

The present invention relates to the manipulation of protein conformation in the protection and release of chemical compounds. That is, the present invention is based on the formation of higher-order structures that proteins assume under various salt, solvent and pH condition so as to protect chemical compounds and/or control the release thereof in *in vitro* or *in vivo* environments. Under physiologic conditions, proteins (polypeptide chains) normally do not exist as extended linear polymer chains. A combination of molecular forces, hydrogen bonding, hydrophilic and hydrophobic interactions, cause the peptide chains to fold up into structure that can either be organized (helices, beta pleated sheets, etc.) or more random. The thermodynamic rules that describe the details of this folding can be complex, for example in the unique formation of an enzymatic site.

The present invention is based upon the utilization of the natural tendency of carefully designed synthetic proteins to fold up, in order to provide protection for sensitive compounds and also provide the engineered release of these compounds. These new products provide important advantages in multiple applications.

SUMMARY OF THE INVENTION

In one embodiment, the invention relates to a method of protecting a chemical compound from degradation comprising combining the chemical

compound with an amino acid or carbohydrate polymer. The amino acid may be, for instance, a glutamic acid polymer or a glutamic acid/tyrosine copolymer. In a preferred embodiment, this chemical compound is iodothyronine or another known thyroid hormone.

In another embodiment, the invention relates to a pharmaceutical composition comprising an active ingredient that has been combined with an amino acid polymer and a pharmaceutically acceptable excipient. The amino acid of the pharmaceutical composition may be, for instance, a glutamic acid polymer, or a glutamic acid/tyrosine copolymer. In one embodiment, the active ingredient of such pharmaceutical composition is L-Dopa or a compound for treating metabolism- or menopausal-associated disorders. In a preferred embodiment, this active ingredient is iodothyronine or another known thyroid hormone.

In yet another embodiment, the invention relates to a method of protecting a chemical compound from degradation comprising manipulating the higher order structure of a synthetic protein and combining said chemical compound with the protein.

In yet another embodiment, the invention relates to a method of controlling the release of a chemical compound comprising manipulating the higher order structure of a synthetic protein and combining said chemical compound with the protein.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

In one embodiment, the present invention relates to polypeptides containing glutamine (an essential amino acid). Monomeric glutamine is an unstable amino acid that can degrade to toxic components under normal cell

culture conditions. However, according to the present invention synthetically produced polypeptides containing glutamine may be used as a nutritional source of stable glutamine. For example, it has been shown that co-polymerization of glutamic acid with glutamine provides a stable form of glutamine.

Stabilization of chemical compounds (that may ordinarily unstable either *in vivo* or on the shelf) in the pharmaceutical compositions of the present invention results from higher order folding of the protein or carbohydrate structure under physiologic conditions. For example, slow proteolytic digestion of the protein in provides a metered release of the chemical compound or active ingredient.

In another embodiment, the polypeptide is made in various lengths to provide a mechanism for sustained release (which may be performed in combination with the manipulation of the higher order structure, as described below). In such an embodiment, the varying length of the polypeptide mixture containing the drug of interest will extend the release of the therapeutic unit from the polypeptide chain, thereby providing a means whereby the frequency of dosing may be reduced. The cause of this extended release is that different length polypeptides will have different rates of hydrolysis. In addition, longer polypeptides will naturally tend to take longer to digest, and thus release the drug of interest. In this way, a mixture of polypeptides of different lengths will promote release throughout an extended period of time. Alternatively, the therapeutic agent may be protected by placing it in a carbohydrate polymer chain of varying lengths, which will similarly promote extended release of the agent.

In a preferred embodiment, the drug to be protected is L-thyroxine, iodothyronine, reverse T3 (rT3) or a related compound, containing more or fewer iodine atoms. The polypeptide in such a case will contain one or more units of

L-thyroxine, iodothyronine or related compounds and be of varying length, for example from about 5 to about 400 amino acids. In other preferred embodiments, the polypeptide of varying lengths may include glutamine, steroids or estrogen.

By providing protein or carbohydrate structures to carry chemical compounds or active ingredients, which are preferably bonded to and part of polymer assemblies of these protein or carbohydrate structures, the folding of the polymers around the active ingredient provides one means of stabilization of the compound or ingredient. In addition, the chemical compound or active ingredient may be stabilized simply by its attachment to one or more carbohydrate or amino acid monomers. By providing a series of polymers of different length which contain the chemical compound or active ingredient, it is possible to make a pharmaceutical composition wherein the different lengths of polymers release the chemical compound or active ingredient at different times, according to the rate at which the polymers are broken down *in vivo.* Having polymers in different lengths will effectively provide a means of time release of the chemical compound or active ingredient. Preferably, the lengths of these polymers (from 5 to about 400) will be sufficient to provide extended shelf life.

Such polymers of amino acids or carbohydrates, along with one or more units of the chemical compound or active ingredient, may be made by methods well known in the art for polypeptide and polycarbohydrate chains. The chemical compound or active ingredient may be inserted either randomly or a designated position during synthesis.

In another embodiment, carefully designed protein conformations are used to protect and release chemical compounds. Certain synthetic polypeptides provide pH and thermodynamic-dependent release of sensitive chemical entities. These producers are especially effect as pH-dependent

dispersants (e.g., in tablet formation) that would selectively release in the stomach or in the small intestine, depending on the specifics of the polypeptide design. Prior to release, chemical entities would be stable due to inclusion in the higher order structure of the polymer and sequestration from O_2, moisture, and other degradants. The careful design of the polymers with hydrophobic pockets (e.g., by inclusion of hydrophobic amino acids like tyrosine) will selectively internalize other hydrophobic molecules for slow release and protection from degradation.

The present invention includes methods and compositions in which the higher order structure of the polypeptide carrier is manipulated such that the resulting polymer has either enhanced or decreased stability for a given set of conditions. One method of manipulating the higher order structure of a protein is by altering its composition, either randomly or with predetermined sequences. It is well known that altering the composition of a polypeptide will alter its three dimensional structure and therefore its stability. It is well within the capabilities of those with ordinary skill in the art to generate a wide range of polypeptides with different random compositions and then screen for, among other things, stability as measured by rate of degradation and/or alteration of tertiary structure through known methods. In addition, there is a wealth of information on known polypeptide structures known to those of skill in the art; the stability of these proteins, especially those that are stable at extreme conditions of temperature and pH, are well-known and studied. The sequences of these known proteins can be adapted and used as carriers, and their structures adapted through random or targeted mutagenesis to decrease or increase stability.

The enhanced shelf life and controlled release of certain drugs is advantageous to the treatment of various disorders, including Parkinson's

disease and hypothyroidism, diseases of the metabolism and conditions associated with menopause.

The following examples are for illustration only and are not extended to limit the invention in any way.

EXAMPLES

Example 1 - Preparation of Polymeric Glutamic Acid (Poly Glu)

The N-Carboxyanhydride of gamma-benzyl glutamic acid (Hglu NCA) is prepared by the method of Daly and Poche (Tet. Letters. Vol. 29, p. 5859, 1988), incorporated herein by reference. For polymerization, the Hglu NCA is prepared as a 10% solution in Tetrahydrofuran (THF) and coupling is initiated by the addition of NaOMe or organic amine, like t-butylamine. When polymerization is complete (e.g., 24 hr.), the benzyl polymer (Poly Bglu) is precipitated by the addition of a suitable solvent, like petroleum ether, and the product dried *in vacuo.* De-benzylation of the polymer with HBr follows the method of Katchalski and Sela (J. Amer. Chem. Soc., Vol. 75, p. 5284, 1953), incorporated herein by reference. The final product (Poly Glu) is obtained as a white, dry powder in about 80% yield and may be further purified, for example, by acid precipitation from aqueous solution.

Example 2 - Preparation of Glutamic Acid/Glutamine Co-Polymer (Poly Glu/Gln)

The Poly BGlu is treated with Hexamethyldisilazane (HMDS, CAS 999-97-3) in THF to form the de-benzylated, silazane-derivatized polymer. This polymer is converted to any desired ratios of glutamic acid and glutamine by a combination of hydrolysis (to form the glutamine amide group) and treatment with HBr (to form the glutamic acid carboxyl group). Alternatively, Poly BGlu may be partially treated with trimethylsilyliodide (TMSI), followed by HMDS and

hydrolysis to get a desired combination of Glu/Gln in the polymer. Tablets suitable for oral treatment are prepared by direct compression.

Example 3 - Preparation of Glutamic Acid/Tyrosine Copolymer (Poly Glu/Tyr)

PBLG is prepared as in Example 1 except that desired amounts of Tyrosine-NCA are incorporated in the polymerization. The polymer is debenzylated in HBr to form the product containing any desired ratio of Glu/Tyr, as a "block" polymer. Alternatively, the Tyr-NCA can be added after 2 Hrs. (for example) of polymerization of the Glu-NCA alone, to obtain polymers enriched in Tyr in the polymer termini. Amino acids other than Tyr can be used to advantage.

Example 4 - Tablet Formation Using Poly Glu

Combinations of Poly Glu and other powdered substances can be prepared by blending a desired amount of Poly Glu and the substance, followed by direct compression to form tablets, if desired. For example, 100 mg of aspirin is combined with 100 mg of Poly Glu and the combination formed into a tablet by direct compression. Additional excipients may be used to advantage, for example to aid in blending or tablet formation.

Example 5 - Treatment for Inflammation

The product of Example 4 is used as an oral preparation for the treatment of inflammation.

Example 6 - Hydrophobic Inclusion by Co-Precipitation with Poly Glu-Tyr

Poly Glu/Tyr as prepared in Example 3 is co-dissolved in THF with a desired amount of Levo-Dopa (L-Dopa). With active stirring, water is slowly

added to co-precipitate the polymer and the monomeric L-Dopa. The precipitate is isolated by centrifugation or filtration and the product dried, e.g., by freeze drying. The product can be formulated and compressed into tablets.

Example 7 - Treatment of Parkinson's Disease

The product of Example 6 is given as an oral preparation to patients with Parkinson's disease, as an anti-Parkinson therapy.

Example 8 - Treatment of Glutamine Deficiency

The product of Example 2 is utilized in humans and other mammals as an effective oral treatment for glutamine deficiency.

Example 9 - *In vitro* Stability of Glutamine as a Component of Poly Glu/Gln

The product of Example 2 was tested for stability and compared to monomeric glutamine by incubation in 0.2 M phosphate buffer at pH 9.0 as described by Price and Greenstein (J. Biol. Chem., Vol. 180, p. 209, 1949), incorporated herein by reference. Glutamine in the polymer was found to be stable for over 12 Hrs. at 37°C, while monomeric glutamine showed extensive degradation to ammonia and pyrrolidonecarboxylic acid, under the same conditions.

Example 10 - Use of Poly Glu/Gln as a Synthetic Serum Extender in Humans

Poly Glu/Gln is useful as a serum substitute and serum extender in humans and other mammals. The product is prepared as a sterile, 0.5% solution in 0.15 N NaC1 and is administered parenterally (I.V.) as needed.

WE CLAIM:

1. A method of protecting a chemical compound from degradation comprising combining said chemical compound with an amino acid polymer.

2. The method of claim 1, wherein said amino acid is a glutamic acid polymer.

3. The method of claim 2, wherein said amino acid is a glutamic acid/tyrosine co-polymer.

4. The method of claim 1 wherein said chemical compound is a thyroid hormone.

5. The method of claim 4 wherein said thyroid hormone is selected from the group consisting of L-thyroxine, iodothyronine, and reverse T3.

6. A pharmaceutical composition comprising an active ingredient that has been combined with an amino acid polymer and a pharmaceutically acceptable excipient.

7. The pharmaceutical composition of claim 6, wherein said amino acid is a glutamic acid polymer.

8. The pharmaceutical compression of claim 7, wherein said amino acid is a glutamic acid/tyrosine co-polymer.

9. The pharmaceutical composition of claim 6, wherein said active ingredient is L-Dopa.

10. The pharmaceutical composition of claim 6, wherein said active ingredient is a thyroid hormone.

11. The pharmaceutical composition of claim 6, wherein said thyroid hormone is selected from the group consisting of L-thyroxine, iodothyronine, and reverse T3.

12. A method of protecting a chemical compound from degradation comprising manipulating the higher order structure of a synthetic protein and combining said chemical compound with said protein.

13. A method of controlling the release of a chemical compound comprising manipulating the higher order structure of a synthetic protein and combining said chemical with said protein.

14. The method of claim 3 wherein the amino acid polymer is a mixture polypeptides of varying lengths.

15. The pharmaceutical composition of claim 6 wherein said amino acid polymer is a mixture polypeptides of varying lengths.

16. The pharmaceutical compound of claim 15 wherein said active ingredient is a thyroid hormone.

17. A method of protecting a chemical compound from degradation comprising combining said chemical compound with a carbohydrate polymer.

18. The method of claim 17 in which said chemical compound is incorporated into said carbohydrate polymer during synthesis of the polymer.

19. The method of claim 18 wherein said carbohydrate polymer is a mixture polymers of varying lengths.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61K 51/00, 39/395, 9/20; A01N 25/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.69, 177, 465; 514/773, 777

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	US 5,955,105 A (MITRA et al.) 21 September 1999, column 3, lines 20-38.	1, 4, 6, 10, 17-19
Y	US 4,224,316 A (MOMANY) 23 September 1980, column 1, lines 10-20, column 2, lines 5-55, column 4, lines 45-60, column 5, lines 8-35, column 6, lines 20-26.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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